Cytology and physiology of silverleaf whitefly-induced squash silverleaf

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Squash silverleaf is a disorder of certain cultivars of *Cucurbita* which results from feeding by the silverleaf whitefly, *Bemisia argentifolii* (formerly, *Bemisia tabaci*, biotype B), but not by the sweetpotato whitefly, *Bemisia tabaci* Biotype A (*B. tabaci*-A). Because squash silverleaf aetiology is unknown, we compared the symptomology, cytology and physiology of *Cucurbita pepo* plants fed upon by *B. argentifolii* and *B. tabaci*-A to find the mechanism of squash silverleaf induction. *B. argentifolii* induced rapid-onset, 100% (of upper leaf surface) expression of squash silverleaf regardless of the season, whereas *B. tabaci*-A induced some intervenal chlorosis but did not induce full expression of squash silverleaf. Cytology of silvered tissue revealed large intercellular air spaces between deformed mesophyll palisade cells and the adaxial epidermis. The chloroplast in the palisade cells and the plasmalemma around some of the vascular cells showed minor ultrastructural damage. Cellular autolysis, similar to other homopteran-induced phytotoxemias, was observed in tissue where the whitefly nymphs had fed and this damage was not due to direct stylet penetration. Ultrastructural examination of squash silverleaf-affected plants did not reveal any viral particles or inclusion bodies. Both whiteflies caused reductions in chlorophyll content, but the loss was greater with *B. argentifolii*. Two new intercellular fluid proteins were induced in silvered leaf tissue and the constitutive expression of another protein was suppressed. Enzyme assays performed on the intercellular fluid proteins indicated it exhibited reduced activities of chitinase and peroxidase. Similarities between this insect-induced host response and pathogen-induced responses are discussed.

INTRODUCTION

Squash silverleaf (SSL) is a disorder of unknown aetiology that affects certain cultivars of Cucurbitaceae when infested by the silverleaf whitefly (SLW), *Bemisia argentifolii* (formerly *Bemisia tabaci*, biotype B) [*2*], but not by the sweetpotato whitefly (SPW), *Bemisia tabaci* (Gennadius) Biotype A (*B. tabaci*-A) [*23*]. SSL symptomology includes an initial venal chlorosis of leaves, followed by a progressive acropetal silvering of the

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Abbreviations used in text: dsRNA, double-stranded ribonucleic acid; EM, electron microscopy; IF, intercellular fluid; LM, light microscopy; PAGE, polyacrylamide gel electrophoresis; PR, pathogenesis-related; SLW, silverleaf whitefly; SPW, sweetpotato whitefly; SSL, squash silverleaf.

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intervenal tissue that culminates in complete silvering of the adaxial leaf surface. Fruit from severely affected plants can have a bleached epidermis, poor quality and reduced yields [*25*]. Early observations of SSL suggested environmental factors such as fertilization and irrigation practices and air quality may contribute to the problem [*41*]. In 1989, SSL was attributed to the presence of elevated populations of SPW [*25*].

Two hypotheses were proposed to explain SSL. Bharathan *et al.* [*3*] reported two double-stranded RNAs (dsRNA) at 4·6 and 4·2 kb in leaf extracts from symptomatic plants and suggested this nucleic acid is the causal agent of whitefly-induced SSL. Yokomi *et al.* [*46*] found dsRNA bands at 4·4 and $4{\cdot}0 \times 10^6$ Da in SSL-virulent whiteflies but no dsRNAs were found in symptomatic leaves or plants. They also noted chlorotic spots on the adaxial leaf surface where the whitefly nymphs fed, and silvering only appeared in new leaves that developed following whitefly infestation. When the whiteflies were removed, affected leaves retained their silvering, but new growth returned to normal. Based on these data and negative evidence for the presence of a plant pathogen, Yokomi *et al.* [*46*] suggested that SSL was caused by a translocated toxicogenic factor associated with whitefly feeding.

In the years after the first reports, the virulent whitefly populations and SSL spread quickly across the southern U.S. and cucurbits and other crops began to have unprecedented high populations of the SPW. In 1992, virulent populations of the SPW were shown to be a different biotype [*15*] which is now known as the SLW [*2*].

In this report we present evidence that whitefly-induced SSL is a host-specific response to the SLW. Cytopathology, chlorophyll reduction and root mass decline, symptoms that are analogous to other homopteran-induced phytotoxicoses, as opposed to those caused by plant disease agents, are presented. Whitefly-mediated induction of intercellular fluid (IF) proteins is presented and the similarities between this insect-induced response and pathogen-induced response are discussed.

MATERIALS AND METHODS

Insects and plants

Colonies of the SLW were maintained in an evaporatively cooled greenhouse on eggplant, *Solanum melogena* L. cv. Florida market 10 (Solanaceae); squash, *Cucurbita pepo* L. cv. Dixie (Asgrow Seed Co., Kalamazoo, MI) and pumpkin, *C. pepo* L. cv. Small sugar (W. Atlee Burpee & Co., Warminster, PA) (Cucurbitaceae). Our colony of SLW is analogous to whiteflies that have been described as *B. tabaci* biotype B and the poinsettia whitefly. These colonies contain dsRNA [*23*, *46*] and esterase patterns that confirm its identity as *B. tabaci*-B (Yokomi, unpublished data). We obtained a colony of the SPW that was collected in 1981 from cotton in California's Imperial Valley (IV-81 [*14*], courtesy of Dr J. E. Duffus, USDA–ARS, Salinas, CA). This SPW colony had esterase patterns indicative of *B. tabaci*-A [*24*] and was maintained on pumpkin and squash. The whiteflies were maintained in 0·16 m^3 isolation cages constructed of splined aluminium frames covered with monofilament nylon screen (300 μm mesh Nitex, Aquatic Eco-Systems Inc., Apopka, FL) and clear acrylic. Access through sleeved openings and constant monitoring prevented colony mixing [*23*]. Whitefly biotype homogeneity was ensured by monitoring SSL, esterase and dsRNA expressions.

Unless specified, all experiments were performed using *C. pepo* L. cv. Small Sugar pumpkin grown under greenhouse conditions in 3·8 l (1 gallon) pots. SSL was rated visually using a six-grade scheme in which non-symptomatic tissue was 0. The progressive acropetal silvering of the veins and areolas was rated 1–4, and complete leaf-blade silvering was assigned a rating of 5 (100% expression of the upper leaf surface) [*34*].

Light and electron microscopy

Twenty-five adult whiteflies were confined to the first or second true leaf on 2-week-old pumpkin plants in clip cages [*44*] and allowed to feed and oviposit. The adults and the clip cage were removed after 5 days and the leaf was checked for oviposition and the plant was subsequently isolated in the greenhouse. Plants were harvested after 3 weeks when most of the immature whiteflies had entered the third or fourth instar stage. Uninfested control and silvered leaves of the same relative age were cut into 1 mm^2 pieces under 5% glutaraldehyde–4% paraformaldehyde in 0·1 M cacodylate buffer followed by fixation with agitation for a minimum of 2 h. Fixed tissue was rinsed twice in fresh buffer, osmicated for 2 h in 2% aqueous OsO_4, dehydrated in ethanol series and embedded in LR White acrylic plastic [medium grade for light microscopy (LM) and hard grade for electron microscopy (EM)]. Thick sections (1 μm) were stained with 0·1% acid fuchsin and methylene blue; thin sections (0·1 nm) were post-stained [*30*] and viewed on a Philips 201 transmission EM at 60 kv. Leaf tissues displaying the chlorotic spots associated with the immature feeding site were also processed for cytology and compared to control tissues of the same relative age.

Chlorophyll determinations and biomass

Pumpkin leaves displaying grade 1–5 SSL were collected from an unsprayed greenhouse infested with the SLW. The chlorophyll content from these leaves was compared to those grown under similar greenhouse conditions without whiteflies. In the first tests, the effects of direct feeding were not separated from the translocated effect at the leaf apex because the whiteflies had free access to the entire foliar canopy. To account for translocated effects, tests were performed where whiteflies were confined to the lower leaves in clip cages as previously described. Individual leaves were weighed immediately after harvest and their area determined using a surface area meter (LI-COR Inc., Lincoln, NE). Six millimetre diameter samples (10–20 mg total) were collected with a paper punch from four separate locations on each leaf and suspended in N,N-dimethylformamide (10 mg tissue ml^{-1}). Chlorophyll concentration was determined as described by Moran [*31*]. Root masses were recorded as wet weights.

Intercellular fluid extracts

Pumpkin leaves were subdivided into three groups: (i) older leaves that were closer to the plant base, distal to the whitefly-infested leaves, and proximal to the leaves expressing SSL; (ii) expanding and fully expanded leaves with SSL symptoms (typically from 2–3 leaves distal to leaves infested with whitefly nymphs to two nodes from the plant apex; and (iii) new leaves which were less than 8 cm^2 and too young to display SSL. Midribs and main veins were removed and the lamina were cut into

2 cm^2 pieces. The leaf pieces were submerged in ice-cold isotonic buffer [*33*] and agitated under vacuum (650 mm Hg) for 30 s. The vacuum was released and this cycle repeated twice. The leaf sections were blotted dry with paper towels, rolled, placed in 10 ml Disposa-Columns (BioRad Laboratories Inc., Melville, NY), and spun at 1000 r min^{-1} for 10 min using a Sorvall SA600 angle rotor at 4 °C. A straw-coloured IF was collected and its protein concentration determined [*5*]. IF from each category was pooled for the assays. Electrophoresis was performed using 16 $cm^2 \times 1{\cdot}5$ mm thick, 7–15% linear acrylamide gradient gels with a 3·9% stacking gel. IF samples containing 50 μg of protein were mixed 2:1 v/v with sample loading buffer and heated for 4 min at 95 °C. SDS-polyacrylamide gel electrophoresis (PAGE) was performed at 30 mA per gel for 4–8 h at 15 °C. Gels were stained with colloidal Coomassie brilliant blue G250 (ISS, Natick, MA) and scanned on a Shimadzu model CS 9000 densitometer using a 633 nm laser source.

IF from pumpkin infected with zucchini yellow mosaic virus (ZYMV), a potyvirus, was included in our experiments to compare with tissue exposed to the whiteflies.

Enzyme assays of IF

Chitinase activity in the IF was measured radiometrically using chitin prepared by acetylation of shrimp shell chitosan with [^{3}H]-acetic anhydride [*29*, *32*]. Peroxidase activity of the IF protein was determined by incubating the gel immediately after PAGE in 0·25% (w/v) *o*-dianisidine in 0·1% (v/v) H_2O_2 in K_2HPO_4 (50 mM, pH 7·0) and activity was determined by dark brown bands in areas where peroxidase was present [*35*].

RESULTS

Symptomology

The SLW consistently induced rapid-onset (9–14 days), grade 5 (100%) SSL throughout the 16-month experimental period. In contrast, the SPW induced only grade 0–1 intervenal chlorosis from October–April. As photoperiod and greenhouse temperatures increased during a 3-month period (June–August), this chlorosis increased to grade 2 but was never accompanied by leaf silvering symptoms. By September and thereafter, SPW-induced chlorosis returned to the 0–1 level as seen in the previous fall and winter. Hence, our data showed that the SLW could induce full expression of SSL (grade 5), whereas the SPW was capable of inducing only symptoms equivalent to early expression (up to grade 2) of SSL but never full SSL regardless of season.

Anatomy of SSL

Grade 5 SSL leaf tissue displayed macroscopic changes in cell organization which included large air spaces between the adaxial epidermis and a deformed layer of palisade cells which was readily seen with the light microscope (Fig. 1). In Dixie squash which has multiseriate palisade tissue, the disorganization of the palisade layer was less severe, and the subepidermal air space was similar to that for the silvering disorder of *Cucurbita* [*6*].

The ultrastructure of the internal organelles in most of the mesophyll cells in silvered pumpkin tissue and those in the control tissue appeared unaffected. In the deformed

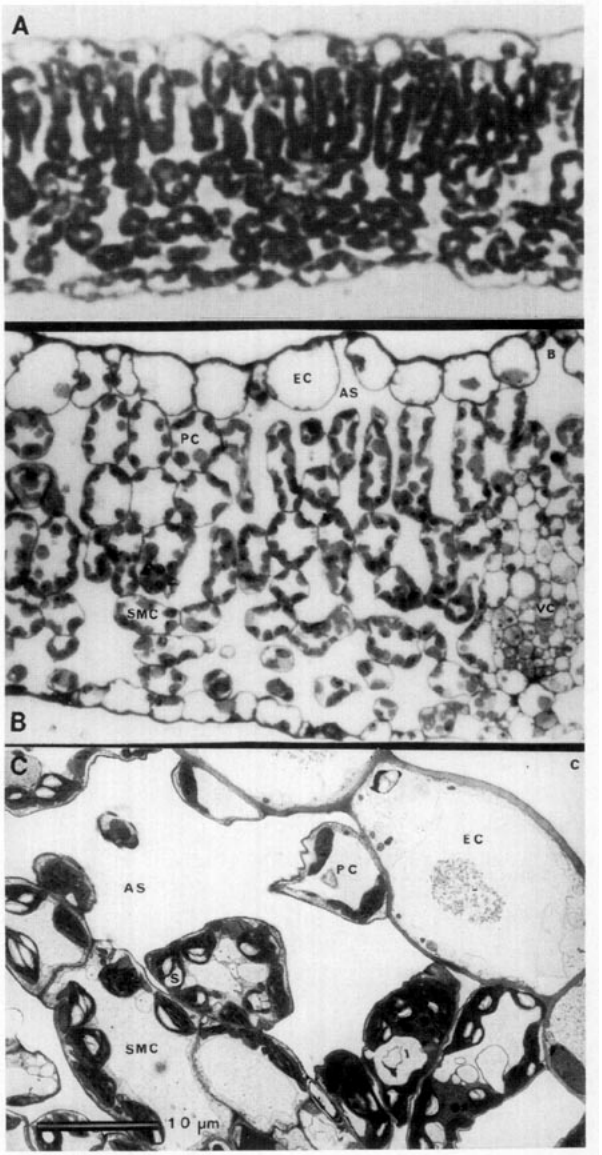

FIG. 1. (A) Thick (1 µm) sections of a control pumpkin leaf showing the cell morphology and the density of chloroplasts in normal mesophyll (×200). (B) Thick section of a silvered pumpkin leaf. The palisade cells (PC) are deformed and surrounded by air spaces (AS). EC, epidermal cells; SMC, spongy mesophyll; VC, vascular bundle (×200). (C) Electron micrograph of pumpkin leaf showing the air space and the deformed palisade cell responsible for the leaf silvering in squash silverleaf. Note that although some palisade cells are deformed, the epidermal cells and the cells of the spongy mesophyll exhibit normal internal architecture and that many of the chloroplasts contain starch (S) (×2000).

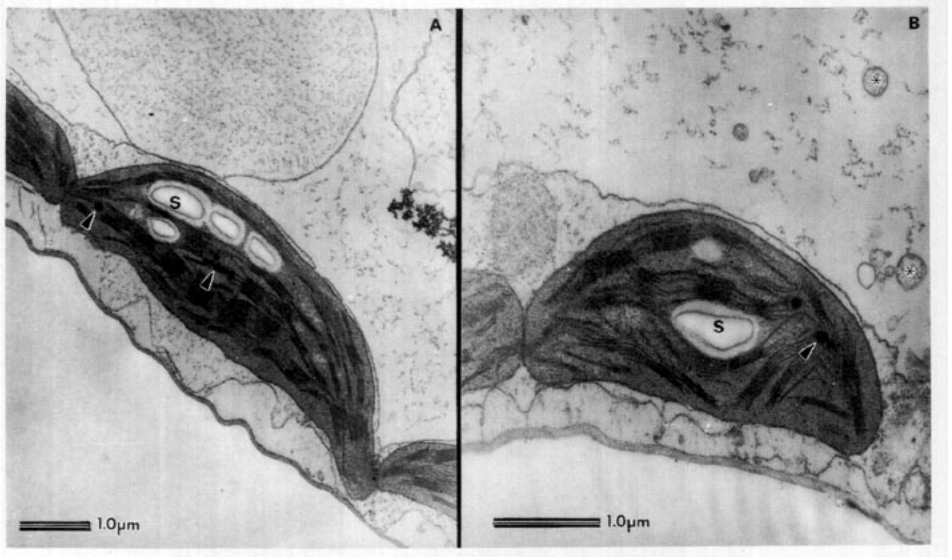

FIG. 2. (A) Chloroplasts from control leaves (× 10000). (B) Chloroplasts from SSL deformed palisade cell. The rounding of the chloroplast and the vesiculation were typical of the cells affected by squash silverleaf. Arrows, plastoglobuli; S, starch; asterisk, vesiculation (× 15000). Reproduced here at 95%.

palisade cells of pumpkin leaf tissue, the mitochondria, Golgi, endoplasmic reticulum, nucleus and their associated membranes appeared normal. Some chloroplasts within the deformed palisade cells were rounded, the thylakoid membranes were moderately disorganized, and the envelope membrane showed signs of deterioration. Otherwise, these chloroplasts were identical to the control with regard to starch accumulation and the deposition of plastoglobuli (lipid globules) (Fig. 2).

Mesophyll cells around the vascular bundle of SSL tissues frequently displayed appositions and vesiculation of the plasmalemma (Fig. 3). However, the occasional

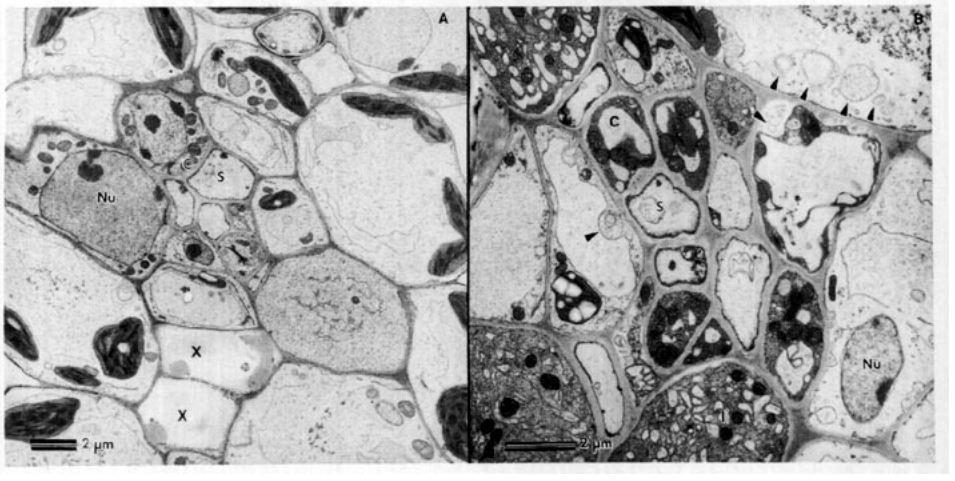

FIG. 3. (A) Cells from the vascular bundle of a control leaf (× 3000). (B) Vascular cells from a silvered leaf. Arrows, vesiculation and apposition of the plasmalemma; C, companion cells; I, intermediary cells; Nu, nucleus; S, sieve elements; X, xylem (× 4500). Reproduced here at 95%.

appearance of similar disturbances in control tissue indicated that this could be due to fixation and exacerbated in SSL tissue by a weakened cell structure.

Plant penetration by whiteflies typically follows an intercellular pathway until phloem is reached. In the case of the SLW, nymphal feeding resulted in a chlorotic spot which was comprised entirely of vacuous mesophyll cells [Fig. 4(A)]. Serial sectioning

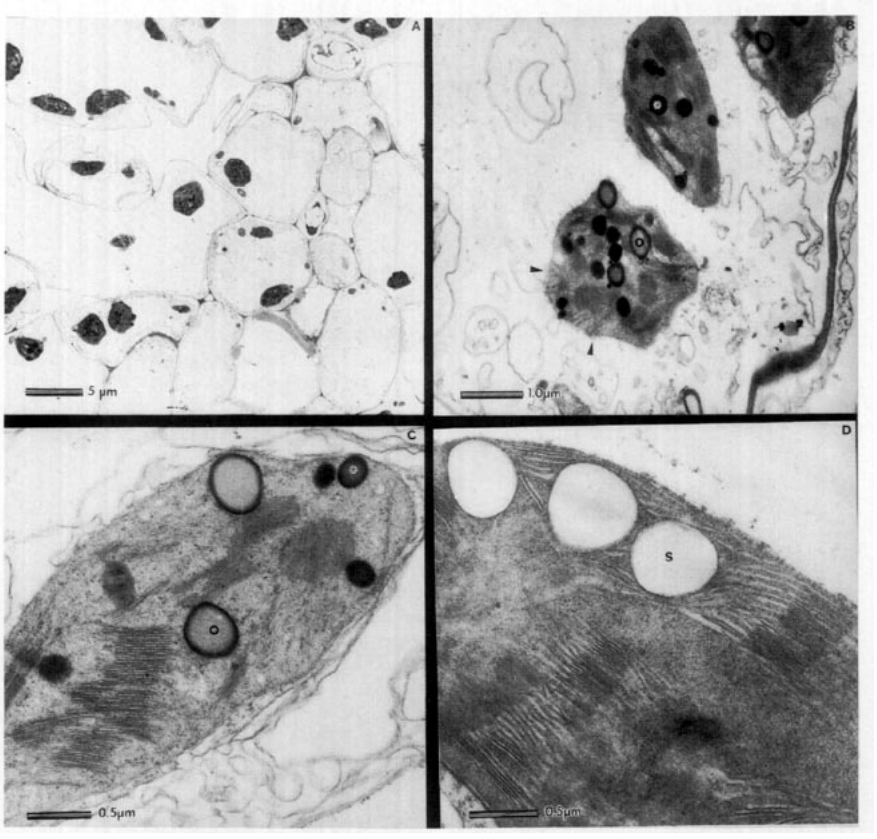

FIG. 4. (A) Vacuous mesophyll cells from the feeding site above the sweetpotato whitefly (SPW), *Bemisia tabaci*-A, nymph. The cell walls are intact but the cytoplasm shows advanced autolysis (×3000). (B) Chloroplasts from a mesophyll cell in the chlorotic spot above the silverleaf whitefly, *Bemisia argentifolii*, nymph. The envelope membranes are degenerate (arrows) and the stroma contains large osmiophilic bodies (asterisk), as well as some opaque bodies with an electron-dense perimeter (O) (×10000). (C) Chloroplast from the feeding site above a SPW-A nymph. Chloroplasts contained normal plastoglobuli and opaque bodies surrounded by an electron-dense perimeter (O) (×20000). (D) Chloroplast from control leaf tissue that is the same age as the tissue in micrographs (B) and (C). The thylakoid membranes are intact and the principal organelles are electron lucent bodies that stained as if they were composed of starch (S) (×40000).

showed that most of the cell walls were intact and that the damage was not due to direct whitefly stylet contact. Except for the nucleus which usually appeared unaffected, the cells were devoid of organelles other than disintegrating chloroplasts and a profusion of vesicles. Remaining chloroplasts were rounded and the envelope membranes were disrupted. Thylakoid membranes and starch granules were absent, and the stroma contained numerous osmiophilic granules which were of the same

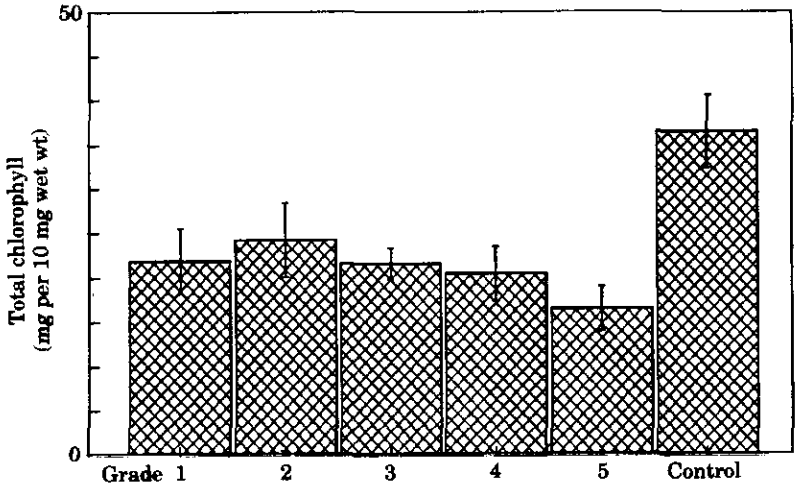

FIG. 5. Reduction in total chlorophyll associated with different grades of squash silverleaf (SSL) induced by the silverleaf whitefly (SLW), *Bemisia argentifolii*. Samples consisted of randomly collected greenhouse-grown pumpkin leaves of unknown age. Values are means ± SD, $n = 15$/grade.

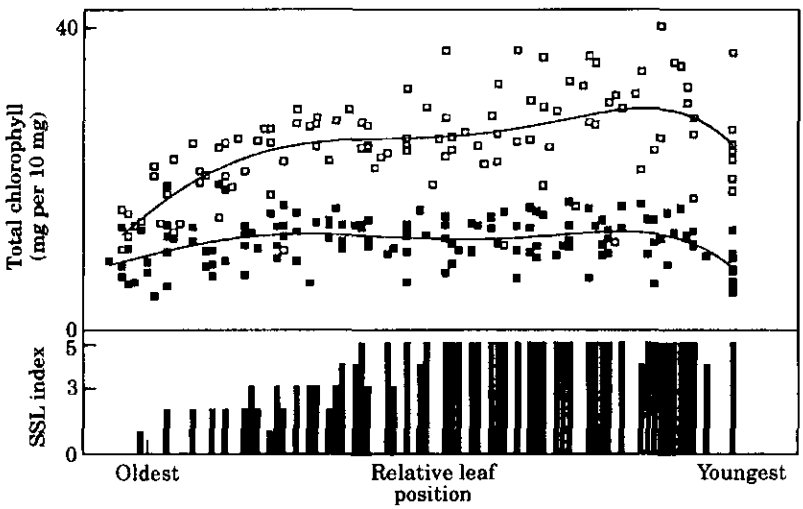

FIG. 6. Reduced total chlorophyll induced by uncontrolled development of the silverleaf whitefly (SLW), *Bemisia argentifolii*, on pumpkin plants. Relative leaf position equalizes differences in the numbers of leaves per plant (n) by placing individual leaves (i) on a scale ranging from 1–100; $(100/n)i$. Regression lines are second order polynomials from eight plants each; SLW (■) mean chlorophyll = 10·2 ± 9·5, $n = 121$; control (□) mean chlorophyll = 23·5 ± 9·1, $n = 122$.

electron density as plastoglobuli, but four to five times larger than normal [Fig. 4(B)]. Some cells contained large opaque granules surrounded by an electron-dense perimeter. No ultrastructural evidence of viral particles or inclusion bodies was detected in any cell around the feeding sites or in leaf tissue displaying SSL.

The mesophyll cells in the chlorotic spots distal to SPW nymphs displayed intermediate levels of tissue disruption. Vesiculation of the plasmalemma was apparent

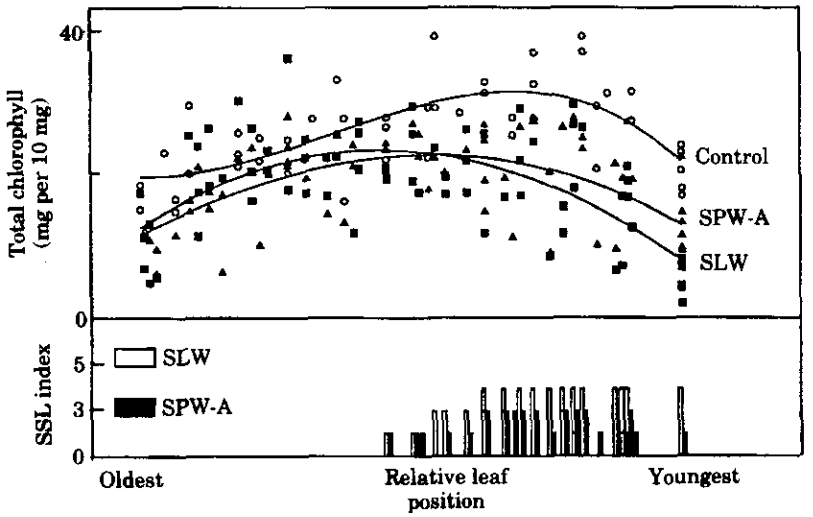

FIG. 7. Reductions in total chlorophyll when the sweetpotato whitefly (SPW-A), *Bemisia tabaci*-A, and the silverleaf whitefly (SLW), *Bemisia argentifolii*, were limited to the first or second true leaf in isolation cages. Chlorophyll reduction coincided with SLW-induced squash silverleaf (SSL) (grade 3–4) and SPW-A-induced venal chlorosis (grade 1–2). Mean values are listed in Table 1.

TABLE 1

Comparison of the effect of the silverleaf whitefly (SLW), Bemisia argentifolii, *and the sweetpotato whitefly (SPW-A)*, Bemisia tabaci-*A, on biomass and chlorophyll pigment levels*

	SLW		SPW-A	
Parameter	Whitefly	Control	Whitefly	Control
Total number of plants	9	8	4	6
Total number of leaves	100	120	77	77
Total number of immature whiteflies	1899	0	4185	0
Mean leaf wet weight (mg)	7·9	7·9	7·5	7·9
Mean leaf surface area (cm^2)	60·21	58·23	55·15	65·85
Mean weight/surface area (mg cm^{-2})	00·2	0·13	0·12	0·12
Mean root wet weight (mg)	5·8*	13·2*	5·1	5·73
Mean chlorophyll A (μg (10 mg)$^{-1}$)	16·61	20·15	21·2	23·71
Mean chlorophyll B (μg (10 mg)$^{-1}$)	5·91	6·72	7·87	8·79
Total mean chlorophyll (μg (10 mg)$^{-1}$)	23·91	26·86	29·05	32·29
Mean chlorophyll A:B ratio	2·88	3·03	2·72	2·74

Paired comparisons of the SLW *v.* control. Means in rows followed by * are significantly different, SAS ANOVA, least significant difference [*39*]. Alpha = 0·05; n = leaf number except in root weight where n = plant number. Since the SPW had little effect on parameters measured, SLW *v.* SPW were not analysed.

and the chloroplast stroma contained small plastoglobuli. The predominant structure in these cells was an opaque deposit bounded by an electron-dense perimeter, similar in size and distribution to the deposits found in the tissues infested with SLW nymphs [Fig. 4(C)].

Mesophyll cells from uninfested control leaves contained a full complement of

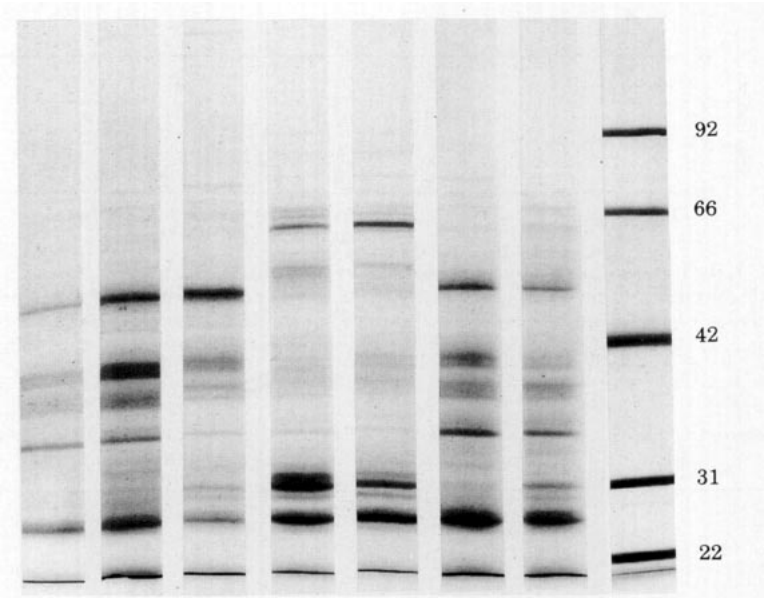

FIG. 8. SDS-polyacrylamide gel electrophoresis of the intercellular fluid (IF) proteins from the silverleaf whitefly- (*Bemisia argentifolii*-) infested, zucchini yellows mosaic virus infected, and control pumpkin leaves. In the lanes containing the IF extracts from grade 3 and grade 5 squash silverleaf (SSL) tissue, two proteins (30800±2·5 and 70000±0·35 Da) were induced and one protein (60000±0·47 Da) disappeared. Molecular weight values are means±SD of seven values; int, intermediate age leaves.

normal cytoplasmic organelles. The chloroplasts contained only small deposits of starch and plastoglobuli, as well as intact thylakoid and envelope membranes [Fig. 4(D)].

Chlorophyll and biomass

Grade 1 SSL was accompanied by a 10–20% reduction in chlorophyll in silvered leaves, whereas grade 5 showed a 40–50% reduction (Fig. 5). This indicated that a significant reduction in chlorophyll content had occurred by the time grade 1 silvering was visible. Figure 6 shows that SLW infestation resulted in a significant reduction in total chlorophyll content in the plant. When whitefly infestations were limited to a single leaf, the reduction in chlorophyll coincided with the induction of SSL in the case of the SLW but only with venal chlorosis in the case of the SPW (Fig. 7). Only leaves that developed after the SLW nymphs initiated feeding developed SSL or showed large reductions in chlorophyll content. Chlorophyll A:chlorophyll B ratios were not

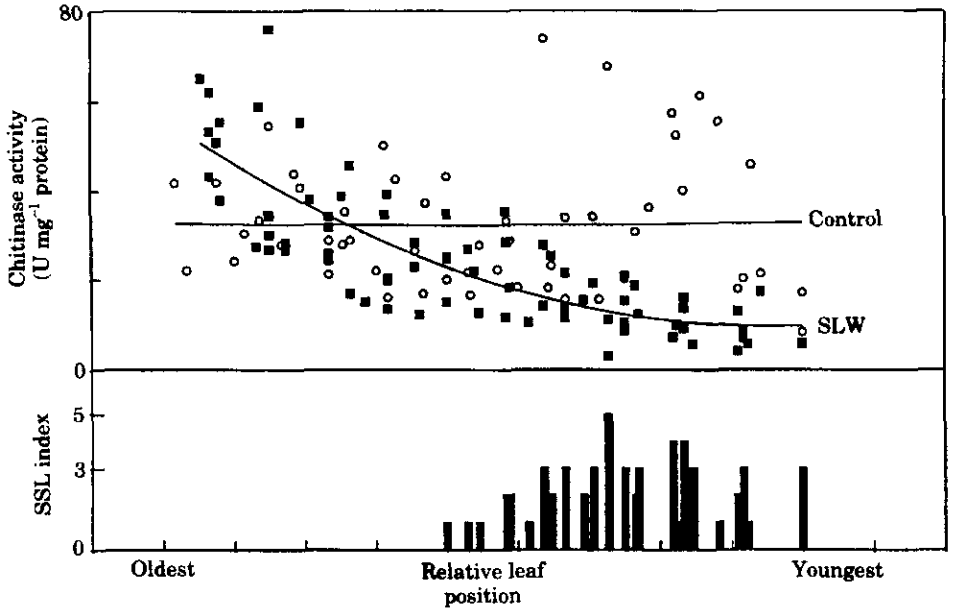

FIG. 9. Reduced chitinase activity in intercellular fluid protein. Data from pumpkin plants 21 days after a 5 day colonization period with 25 adult silverleaf whitefly (SLW), *Bemisia argentifolii*; (■) control plants with no whiteflies (○).

significantly different, and leaf biomass was unaffected as well. The SLW, however, did cause a significant reduction in root mass ($P < 0{\cdot}05$) whereas the SPW did not (Table 1).

IF extracts

SSL symptomology in pumpkin was accompanied by an alteration in the production of IF proteins. Old leaves and immature new leaves contained profiles similar to controls. Visible changes in the density of three protein bands were apparent in the extracts from leaf tissue that expressed SSL (Fig. 8). New proteins appeared with mol. wts estimated to be 70000 and 31000 Da. A prominent 60000 Da protein disappeared. A large standard deviation associated with the estimated mean mol. wt, and laser densitometry, suggested that the 31000 Da band may be composed of more than one protein. Regardless of seasonal effect previously mentioned, the SPW induced some intervenal chlorosis which was accompanied by a slight increase in a 70000 Da protein. Infection with Zucchini yellows mosaic virus caused a slight increase in a 31000 Da protein. When whole leaves were homogenized in acidic (pH 2·8) or basic (pH 8) buffer and extracts electrophoresed, protein patterns were identical to controls. This meant that the new proteins were not detectable in whole leaf extracts and were, therefore, present in small quantities.

Enzyme assays of IF

Chitinase activity in the IF was reduced in relation to SSL expression (Fig. 9). Furthermore, peroxidase activity in the IF from silvered leaves was the same as that in the controls (Fig. 10). Contrary to expectations, these data indicated that the 70000

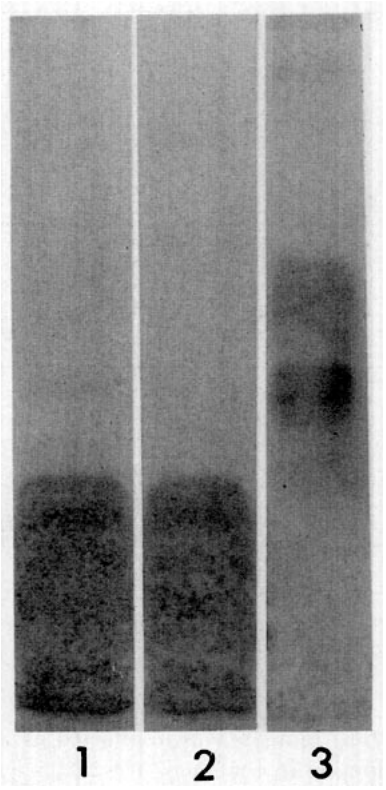

FIG. 10. Peroxidase enzymes for intercellular fluid protein from the silverleaf whitefly (SLW), *Bemisia argentifolii*, silvered Dixie squash leaves (lane 1), non-silvered control (lane 2), and horseradish peroxidase in lane 3 in 7% native polyacrylamide gels.

and 31 000 Da IF proteins which were induced by the SLW were not active enzymes typically induced by a general response to stress factors in squash.

DISCUSSION

Some insects cause phytotoxemias that are away from but vascularly connected to the feeding site in host tissue [*27*, *38*] implying that active molecule(s) are translocatable. The responsible molecules have not been characterised, but two hypotheses exist to explain their effects: (i) molecules similar to microbial toxins occur in the insect saliva are injected into the host plant upon feeding and translocate apically; or (ii) mobile secondary messengers arise from damaged plant cells at the insect feeding site, triggering a series of physiological changes in subsequent plant development.

Although insect salivary toxins or signals have not yet been definitively identified, the induction of plant allelochemicals in response to insect feeding damage is documented [*18*, *37*]. There are numerous examples where a low infestation of phytophagous insects still elicits a significant effect on host plant development (i.e. chlorosis, stunting, leaf deformation and gall formation). Root mass decline, chlorophyll

pigment reduction, induction of premature senescence and reallocation of plant nutrients have been shown to result from phloem-feeding Homoptera [*8*, *16*, *17*, *20*, *45*], pathogens and other stress or damage factors.

Anatomically, SSL is similar to squash leaf silvering [*7*]. Leaf silvering has been attributed to differential growth rates between the palisade mesophyll and the epidermal cells in cucurbits and other plants. In the case of apple leaf silvering, a fungal pathogen, *Chondrostereum purpureum* Pouzar, was implicated in the production of translocated substance(s), probably hydrolytic enzymes (pectinases) or their products (oligosaccharides), which were considered the causal elements [*28*]. Since homopterans produce salivary pectinase and cellulase [*11*, *27*], oligosaccharides released as a result of salivary enzyme activity, as well as from physical wounds and fungal pathogens, have been implicated in a variety of induced systemic plant responses [*11*, *17*, *37*].

Byrne & Miller [*9*] attributed the increased host range of the poinsettia whitefly (= SLW, *B. argentifolii*) to its ability to process more phloem sap than the cotton strain of the SPW. Some Homoptera become artificial sinks by consuming large amounts of phloem sap, and certain biotypes can increase the amino nitrogen content of the sap, theoretically increasing the fitness of one insect race over another [*38*, *45*]. The increased honeydew production of the poinsettia whitefly may be due to its ability to induce phloem sap translocation. Such nutrient reallocation has been shown to be a plant response to a water deficiency which resulted in reduced root mass [*8*, *16*] similar to that induced by the SLW in our studies. Plant water deficit has been related to the leaf silvering of cucurbits [*7*]; light intensity has been suggested as important in SSL expression [*14*]. The modification of chloroplast ultrastructure in the deformed palisade cells of SSL tissue is similarly encountered as the result of a water-stressed, wilted condition [*21*]. Paris *et al.* [*34*] suggested that silvering may be a plant response that may help reduce desiccation.

The fact that the SLW can induce SSL in certain cultivars of the Cucurbitaceae suggests that silvering is a host-specific symptom. Cytopathological effects at the nymphal feeding site indicate that the SLW possesses a unique ability to induce rapid autolysis in cells of susceptible hosts. This damage was similar to that induced by phytotoxic *Schizaphis graminum* (Rondani) biotype E on susceptible cultivars of wheat, where the principal effects also appeared in cell membranes and the chloroplast [*1*, *38*]. In the case of *S. graminum*, pectin methylesterase and other polysaccharases are well-documented factors in the systemic phytotoxic response of wheat to greenbug feeding [*10*, *11*, *17*].

Our data do not preclude the possibility of a translocated whitefly-borne toxin. Several microbial phytotoxins are known to affect photosynthetic function and reduce chlorophyll concentration without inducing obvious changes in chloroplast ultrastructure [*21*, *40*]. Both whitefly populations in our study caused some chlorophyll reduction, but only the SLW consistently induced full expression of SSL. The weak correlation of chlorophyll reduction and expression of leaf silvering suggested that they may not be closely linked.

The systemic induction of the phytoalexin, coumesterol, in response to aphid feeding has been reported [*11*], but our report is the first to document the induction of IF proteins in response to insect attack. Induction of plant proteins resulting from pathogen infections is a well-known occurrence. Pathogenesis-related (PR) proteins are

plant proteins that are induced in pathological conditions which can include infections by plant pathogens, nematodes, phytophagous insects and herbivores [*43*]. Enzymes that have been identified in the various PR protein families include chitinases and β-1,3-glucanases [*4, 12*]. Many of the PR proteins have been implicated in defensive and resistance mechanisms to plant pathogens [*4, 12*]. In some instances, molecular signals such as oligosaccharides and ethylene [*19, 36*] are thought to activate the production of defensive proteins. These signals can be generated as a result of pathogen pectinase and cellulase digestion of plant cell walls, digestion of pathogen cell wall by PR proteins, and wounding. The two proteins at 31000 and 70000 Da (Fig. 8) that result from SSL appear to meet the criteria for PR proteins. In addition, one of the proteins (31000 Da) also appears to be induced upon Zucchini yellows mosaic virus infection. Chitinases and peroxidases are often induced in cucumber plants infected with various pathogens [*13, 26, 42*] or treated with potassium phosphate [*22*]. We were not able to correlate peroxidase or chitinase activity with either protein. In our experience, this is not unusual with plant proteins that are induced by insect attack. With larval *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae) feeding on citrus roots, we found that immunodetection was necessary to reveal the presence of induced chitinases; activity measurements alone were not sufficient to detect elicited enzymes (unreported). Further, in some citrus cultivars, a decrease in root chitinase, β-1,3-glucanase, and chitosanase activities was found following insect herbivory (unreported).

It has also been suggested that biotypic variation and phytotoxicity in some members of the Homoptera may be the result of extragenomic inheritance arising from the insect's prokaryotic endosymbiotes [*10, 38*]. The similarity of the cytopathological and biochemical effects of the SLW with several other phytotoxic sap-sucking insects and certain plant pathogens supports the hypothesis that the effector responsible for the phytotoxemia may be related to bacterial endosymbiotes found within the insect.

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